

(Z,Z)-6,9-HENEICOSADIEN-11-ONE: MAJOR SEX
PHEROMONE COMPONENT OF PAINTED APPLE MOTH,
Teia anartoides

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Abstract—(Z,Z)-6,9-Heneicosadien-11-one (Z6Z9-11-one-21Hy) was identified as the major sex pheromone component of the painted apple moth (PAM), *Teia anartoides* (Lepidoptera: Lymantriidae), on the basis of (1) comparative gas chromatographic-electroantennographic detection (GC-EAD) analyses, GC-mass spectrometry (MS), high-performance liquid chromatography (HPLC)-MS, and HPLC-UV/visible spectroscopy of pheromone gland extracts and authentic standards; (2) GC-EAD analyses of effluvia of calling females; and (3) wind tunnel and field trapping experiments with a synthetic standard. In field experiments in Australia, synthetic Z6Z9-11-one-21Hy as a single component attracted male moths. Wind tunnel experiments suggested that a 4-component blend consisting of Z6Z9-11-one-21Hy, (6Z,9R,10S)-cis-9,10-epoxy-heneicosene (Z6-9R10S-epo-21Hy), (E,E)-7,9-heneicosadien-6,11-dione (E7E9-6,11-dione-21Hy), and 6-hydroxy-(E,E)-7,9-heneicosadien-11-one (E7E9-6-ol-11-one-21Hy) (all present in pheromone gland extracts) might induce more males to orient toward, approach, and contact the source than did

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Z6Z9-11-one-21Hy as a single component. Additional experiments are needed to determine conclusively whether or not Z6-9R10S-epo-21Hy, E7E9-6,11-dione-21Hy, and E7E9-6-ol-11-one-21Hy might be minor sex pheromone components of PAM. Moreover, attractiveness of synthetic pheromone and virgin PAM females needs to be compared to determine whether synthetic pheromone could replace PAM females as trap baits in the program to monitor eradication of exotic PAM in New Zealand.

Key Words—Lymantriidae, *Teia anartoides*, painted apple moth, sex pheromone, (Z,Z)-6,9-heneicosadien-11-one, (Z,E)-6,8-heneicosadien-11-one, (6Z,9R,10S)-cis-9,10-epoxy-heneicosene, (E,E)-7,9-heneicosadien-6,11-dione; 6-hydroxy-(E,E)-7,9-heneicosadiene-11-one.

INTRODUCTION

The painted apple moth (PAM), *Teia anartoides* Walker (Lepidoptera: Lymantriidae), is endemic to southeastern Australia (Common, 1990; Edwards, 1996). Caterpillars cause minor damage to a wide range of trees and shrubs (Edwards, 1996), especially Acacias (*Acacia* spp.), and many other native and introduced plants, including cultivated crops and forest trees such as *Pinus radiata* (Pinaceae) (Common, 1990).

In 1999, PAM caterpillars were discovered in Auckland, New Zealand (Ridley, 1999). Realizing the threat of this exotic moth to New Zealand's native plants, agricultural, horticultural, and silvicultural industries as well as international trade, New Zealand's government initiated a program to eradicate PAM. Progress in this ongoing program is monitored by traps baited with laboratory-reared virgin PAM females. Continuous trap captures of PAM males indicate the persistence of residual PAM populations. Synthetic female pheromone for use as trap bait would facilitate monitoring of the eradication program, and prompted attempts to identify the PAM pheromone.

A number of compounds have been reported as possible sex pheromone components of PAM, including (Z)-6-heneicosen-11-one (Suckling et al., 2002; Muto and Mori, 2003), (Z,E)-6,8-heneicosadien-11-one (Jury et al., 2003; Muto and Mori, 2003), (6Z,9R,10S)- and (6Z,9S,10R)-cis-9,10-epoxy-heneicosene, as well as (6Z,9R,10S)- and (6Z,9S,10R)-cis-9,10-epoxy-eicosene (Muto and Mori, 2003). None of these studies report bioassay data that demonstrate attraction of PAM males to either one or all of these components. Here, we report chemical analyses, and wind tunnel and field experiments, demonstrating that (Z,Z)-6,9-heneicosadien-11-one (Z6Z9-11-one-21Hy) is the major sex pheromone component of female PAM.

METHODS AND MATERIALS

Experimental Insects. PAM caterpillars were reared on gypsy moth diet (Bell et al., 1981) (photoperiod: 14L:10D; temperature: 24°C; relative humidity:

55–60%) at the USDA Quarantine Facility in Newark, DE, USA, and at Forestry Research, Rotorua, New Zealand. Male and female pupae were sent by courier to Simon Fraser University (SFU), and were kept in SFU's Global Forest Quarantine Facility.

Collection of Pheromones. Abdominal tips with pheromone glands of calling, 2- to 3-d-old females were removed and extracted in HPLC-grade hexane. To obtain effluvia of calling females, 30 females were placed in a Pyrex glass chamber maintained at a photoperiod of 14L:10D and a temperature of 25°C. A water aspirator was used to draw humidified, charcoal-filtered air at 80 ml/min through the chamber and through a glass column (6 × 30 mm) filled with Porapak Q (50–80 mesh, Waters Associated Inc. Milford, MA, USA). To minimize isomerization of volatiles, the Porapak Q trap was kept at 5°C. After 48 hr, absorbed volatiles were desorbed with 2 ml of redistilled pentane.

Analyses of Pheromone and General Instrumentation. Aliquots of 0.1 female equivalent (FE) of pheromone gland extract, or 30 female-hour equivalents (FHE = all volatiles released by 30 PAM females during 1 hr of aeration) of Porapak Q

190–600 nm), and an Agilent SB-C18 column (2.1 × 75.0 mm with 3.5 μm packing) fitted with a guard column (2.1 × 12 mm) consisting of XDB-C8 (5 μm particle size). The injection volume was 0.1 μl for both synthetic standards and pheromone extract. The mobile phase consisted of an isocratic solvent system, with 10% of solvent A [H₂O (95%): MeOH (5%)] and 90% of solvent B [MeOH (100%)] at a flow rate of 0.25 ml/min. MSD scan range and time, respectively, were 100–400 amu and 2.82 sec, with the fragmentator at 200 volts. The N₂ nebulizer pressure was 20 psi, and the flow rate of the drying gas 10 l/min at a temperature of 350°C. The capillary voltage was set at 3.5 kV. Samples were processed by atmospheric pressure electrospray (APES), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photo ionization (APPI).

Nuclear magnetic resonance (NMR) spectroscopy of synthetic compounds was conducted on a Varian AS500 spectrometer at 499.77 MHz for ¹H NMR and 125.68 MHz for ¹³C spectra. ¹H chemical shifts are reported as parts per million [ppm, relative to TMS (0.00 ppm)]. Infrared (IR) spectroscopy of synthetic compounds was conducted on a Bomem MB Series FTIR spectrometer. Elemental analyses of synthetic chemicals were performed with a Carlo Erba Model 1106 elemental analyzer.

Source of Synthetic Standards. (Z,Z)-6,9-Heneicosadiene (Z6Z9-21Hy), (9R,10S)-*cis*-9,10-epoxy-(Z)-6-eicosene (Z6-9R10S-epo-20Hy), (9S,10R)-*cis*-9,10-epoxy-(Z)-6-eicosene (Z6-9S10R-epo-20Hy), (9R,10S)-*cis*-9,10-epoxy-(Z)-6-heneicosene (Z6-9R10S-epo-21Hy), and (9S,10R)-*cis*-9,10-epoxy-(Z)-6-heneicosene (Z6-9S10R-epo-21Hy) were donations from Edward W. Underhill (National Research Council of Canada, Plant Biotechnology Institute, Saskatoon, Sask. Canada). (Z)-6-Heneicosen-11-one (Z6-11-one-21Hy) was purchased (Bedoukian, Danbury, CT, USA). (Z,E)-6,8-Heneicosadien-11-one (Z6E8-11-one-21Hy) and (Z,E)-6,9-heneicosadien-11-one (Z6E9-11-one-21Hy) were available from previous work (Gries et al., 1997). (Z,Z)-6,9-Eicosadien-11-one (Z6Z9-11-one-20Hy) and (Z,Z)-6,9-heneicosadien-11-one (Z6Z9-11-one-21Hy) were produced by oxidation of corresponding alcohols available in our laboratory (Gries et al., 2003). Racemic (Z,Z)-6,9-heneicosadien-11-ol (Z6Z9-11-ol-21Hy), and enantiomers thereof, were available from previous work (Gries et al., 2003).

Syntheses. We describe syntheses and report spectroscopic data for (E,E)-7,9-heneicosadien-6,11-dione (E7E9-6,11-dione-21Hy) and 6-hydroxy-(E,E)-7,9-heneicosadien-11-one (E7E9-6-ol-11-one-21Hy), two previously unknown components in pheromone gland extracts of insects. Chemicals obtained from commercial sources were used without further purification unless otherwise indicated. All moisture- and air-sensitive reactions were conducted under argon. Column chromatography refers to flash chromatography using silica gel 60 (230–400 mesh, E. Merck, Darmstadt, Germany) (Still et al., 1978).

6-Hydroxy-(E,E)-7,9-heneicosadien-11-one (7) and (E,E)-7,9-Heneicosadien-6,11-dione (8) (Figure 1). Silylation of 1-octyne-3-ol (1) (Aldrich Chem.

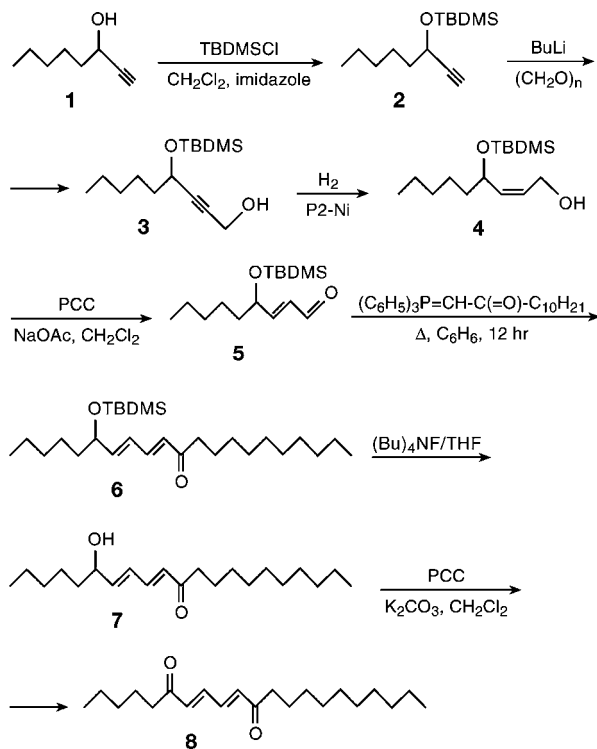


FIG. 1. Scheme for the syntheses of (*E,E*)-7,9-heneicosadien-6,11-dione and 6-hydroxy-(*E,E*)-7,9-heneicosadien-11-one.

Co) and subsequent formylation of silyl ether **2** (99% yield) gave alcohol **3** (94% yield) (Marshall and Zou, 2000). Hydrogenation of **3** (2.75 g, 0.01 mol) with P2-Nickel catalyst (Brown and Ahuja, 1973) afforded alcohol **4** (2.6 g, 96% yield) of which 50% (1.3 g, 4.7 mmol) was oxidized by pyridinium chlorochromate (PCC) (1.60 g) in dichloromethane in the presence of sodium acetate (0.60 g, 7.3 mmol). The reaction mixture was filtered through silica (15 g) with hexane-ether (19:1) as eluent, and solvents were removed *in vacuo*. Without further purification, crude aldehyde **5** (Hayashi, 1990) was refluxed 12 hr in benzene with the stabilized ylid 1-triphenylphosphoranylidene-2-dodecanone. This ylid was obtained (90% yield) by deprotonation (butyllithium) and alkylation (*n*-nonyl iodide) of 1-triphenylphosphoranylidene-2-propanone (Avocado Research Chem. Ltd., Lancashire, UK) (Taylor and Wolf, 1972; Black et al., 1996). Following reflux, benzene was removed *in vacuo*, and unreacted ylid and triphenylphosphine oxide were removed by filtering the resulting mixture through silica (15 g) with hexane-ether (9:1) as eluent. Without further separation, filtrates containing

silylated keto-alcohol **6** were concentrated and treated with a THF solution of Bu_4NF for 2 hr. The reaction mixture containing the desired keto-alcohol **7** (formation of only one geometric isomer observed) was diluted with water and extracted with ether, the ether layer was washed with water and brine, then dried (MgSO_4), concentrated, and purified by flash chromatography (50 g silica) with hexane/ether (3:1) and (1:1) as consecutive eluents. Yield of pure keto-alcohol **7** was 0.30 g (20% based on alcohol **4**, overall yield 17%). M.p. 43°C . Anal. calcd. for $\text{C}_{21}\text{H}_{38}\text{O}_2$ (%): C, 78.28; H, 11.88, found: C, 78.26, H, 12.20. IR (KBr): 3650, 3630, 2955, 2918, 2850, 1686, 1605, 1088, 996 cm^{-1} . ^1H NMR (CDCl_3) δ : 0.90–0.98 (m, 6H), 1.21–1.35 (m, 20H), 1.54–1.64 (m, 4H), 2.55 (t, 2H, $J = 7.6$ Hz), 4.25 (1H, m), 6.13–6.23 (m, 2H), 6.37 (dd, 1H, $J = 15.0, 11.0$ Hz), 7.15 (dd, 1H, $J = 15.5, 11.0$ Hz). ^{13}C NMR (CDCl_3) δ : 14.01, 14.11, 22.56, 22.67, 24.35, 24.94, 29.31 (2), 29.43, 29.48, 29.56, 31.68, 31.88, 37.05, 40.74, 72.09, 127.89, 129.81, 141.49, 145.62, 200.95.

Oxidation of **7** (0.15 g, 0.47 mmol) with PCC (0.15 g, 0.70 mmol) in the presence of potassium carbonate (0.050 g, 0.036 mmol) for 2 hr in dry CH_2Cl_2 afforded diketone **8** (0.14 g, 93% yield based on keto-alcohol **7**). M.p. 76°C . Anal. calcd. for $\text{C}_{21}\text{H}_{36}\text{O}_2$ (%): C, 78.70; H, 11.32, found: C, 79.08, H, 11.12. IR (KBr): 3650, 2955, 2916, 2850, 1678, 1587, 1407, 1216, 1096, 1010 cm^{-1} . ^1H NMR (CDCl_3) δ : 0.86–0.92 (m, 6H), 1.28–1.36 (m, 18H), 1.59–1.67 (m, 4H), 2.60 (t, 4H, $J = 7.5$ Hz), 6.49 (dd, 2H, $J = 11.5, 2.8$ Hz), 7.18 (dd, 2H, $J = 11.7, 2.8$ Hz). ^{13}C NMR (CDCl_3) δ : 13.91, 14.11, 22.45, 22.67, 23.72, 24.04, 29.22, 29.30, 29.40, 29.46, 29.55, 31.38, 31.88, 41.31, 41.35, 135.96, 135.97, 138.79, 138.80, 200.10, 200.11.

Wind Tunnel and Field Experiments. Wind tunnel experiments employed a wind tunnel (160 cm long, 68 cm wide, 70 cm tall) and protocol as developed by Miller and Roelofs (1978). The air speed was 30 cm/sec, and the temperature was 22°C ($\pm 2^\circ\text{C}$). Experiments bioassayed the responses of individually tested 1- to 3-d-old males to filter paper (Whatman International Ltd., Maidstone, England) impregnated with test chemicals in HPLC-grade hexane or acetonitrile. All test chemicals were >95% chemically pure. Six criteria (Table 1) were used to assess the attractiveness of lures. Experiment 1 tested Z6Z9-11-one-21Hy singly and in combination with either Z6-9R10S-epo-21Hy (a), E7E9-6,11-dione-21Hy plus E7E9-6-ol-11-one-21Hy (b), or (a) plus (b). Experiment 2 tested Z6Z9-11-one-21Hy singly and in binary, ternary, and quaternary combinations with Z6-9R10S-epo-21Hy, E7E9-6,11-dione-21Hy, and E7E9-6-ol-11-one-21Hy (Table 1).

Field experiments were conducted in Campbelltown, N.S.W., Australia, in the residential area of Wedderburn (S $34^\circ 09'$, E $150^\circ 49'$) generally in forest types classified as "dry sclerophyll" (Sydney, Hawkesbury sandstone). The area was dominated by trees of *Eucalyptus* spp. with some *Acacia* spp., and stocked with numerous shrubs in formerly disturbed or open field habitats. Delta-type traps made from 2-l milk cartons (Gray et al., 1984) were coated with

TABLE 1. WIND-TUNNEL BIOASSAYS WITH MALES OF PAINTED APPLE MOTH, *Teia anartoides*, RESPONDING TO FILTER PAPERS IMPREGNATED WITH CANDIDATE PHEROMONE COMPONENTS

Exp.	Treatment ^a	Males responding to assessment criteria ^b (N = male moths tested)						
		A	F	O	H	Ap	L	
1	Z6Z9-11-one	(N = 12)	6	6	5	0	0	
	Z6Z9-11-one + Z6-9R10S-epo	(N = 12)	9	9	3	1	1	
	Z6Z9-11-one + E7E9-6,11-dione + E7E9-6-ol-11-one	(N = 12)	8	7	0	0	0	
	Z6Z9-11-one + Z6-9R10S-epo + E7E9-6,11-dione + E7E9-6-ol-11-one	(N = 13)	13	13	13	6	4	
2	Z6Z9-11-one	(N = 13)	8	6	1	0	0	
	Z6Z9-11-one + Z6-9R10S-epo	(N = 13)	6	6	5	4	3	
	Z6Z9-11-one + E7E9-6,11-dione + E7E9-6-ol-11-one	(N = 13)	2	3	0	0	0	
	Z6Z9-11-one + Z6-9R10S-epo + E7E9-6,11-dione	(N = 13)	12	9	8	1	1	
	Z6Z9-11-one + Z6-9R10S-epo + E7E9-6-ol-11-one	(N = 13)	9	8	6	3	2	
	Z6Z9-11-one + Z6-9R10S-epo + E7E9-6,11-dione + E7E9-6-ol-11-one	(N = 13)	11	11	9	5	5	

^aCompound abbreviations as follows: Z6Z9-11-one = (Z,Z)-6,9-heneicosadien-11-one (57 ng = 1 female equivalent of pheromone gland extract); Z6-9R10S-epo = (Z6,9R,10S)-*cis*-9,10-epoxy-heneicosene (7 ng); E7E9-6,11-dione = (E,E)-7,9-heneicosadien-6,11-dione (3.5 ng); E7E9-6-ol-11-one = 6-hydroxy-(E,E)-7,9-heneicosadien-11-one.

^bA = Activation; F = flight; O = 4 orientation towards pheromone lure; H = halfway flight toward lure; Ap = approaching lure; L = landing on lure.

Tanglefoot (The Tanglefoot Company, Grand Rapids, MI, USA) and suspended from trees at a height of 2 m and spacings of 20–25 m in complete randomized blocks, which were separated by 0.1 to 3 km. Traps were baited with a piece of dental cotton roll (10 × 15 mm) (Richmond Dental, Charlotte, NC, USA), which was impregnated with test chemicals just prior (0–2 hr) to the onset (~10:00 am) of experiments. To prevent rearrangement of (labile) test chemicals during storage, they were placed in vials, diluted in solvent (hexane, pentane/ether, or acetonitrile), kept on dry ice, and allowed to warm up to ambient temperature only during the 15–30-min preparation of test lures.

Field experiment 1 tested the major candidate pheromone component Z6Z9-11-one-21Hy (5 µg) singly and in quaternary combination with secondary candidate pheromone components Z6-9R10S-epo-21Hy, *E7E9*-6,11-dione-21Hy, and *E7E9*-6-ol-11-one-21Hy at ratios of 5:5:5:5 or 5:0.5:0.5:0.5. Taking into account that the 4-component blend (5: 0.5: 0.5: 0.5) appeared most attractive, experiment 2 tested Z6Z9-11-one-21Hy (5 µg) singly and in combination with either one or all three of Z6-9R10S-epo-21Hy, *E7E9*-6,11-dione-21Hy, and *E7E9*-6-ol-11-one-21Hy at 10:1 ratios. Considering that Z6-9R10S-epo-21Hy appeared to enhance attractiveness of Z6Z9-11-one-21Hy in experiment 2, experiments 3 and 4 explored whether attractiveness of this 2-component blend could be enhanced by additional components. Experiment 3 tested Z6Z9-11-one-21Hy (5 µg) plus Z6-9R10S-epo-21Hy (0.5 µg) in combination with either *E7E9*-6,11-dione-21Hy (0.5 µg), *E7E9*-6-ol-11-one-21Hy (0.5 µg), or both, whereas experiment 4 tested Z6Z9-11-one-21Hy (5 µg) plus Z6-9R10S-epo-21Hy (0.5 µg) in combination with either Z6Z9-11-one-20Hy (0.5 µg), Z6-11-one-21Hy (0.5 µg), Z6E8-11-one-21Hy (0.5 µg), Z6E9-11-one-21Hy (0.5 µg), Z6Z9-11-ol-21Hy (0.5 µg) or Z6-9R10S-epo-20Hy (0.5 µg). Experiment 5 tested 3 components singly: Z6Z9-11-one (5 or 0.5 µg), the corresponding rearrangement product Z6E8-11-one-21Hy (5 µg), and Z6-9R10S-epo-21Hy (5 µg). In all experiments, lures were replaced after two days without re-randomizing treatment positions.

Trap catch data were analyzed by nonparametric analysis of variance (Friedman's test) followed by comparison of means by Scheffé test (Zar, 1984; SAS/STAT, 1988). In all analyses, $\alpha = 0.05$.

RESULTS

Chemical Analyses. GC-EAD analyses of PAM pheromone gland extract revealed many components that consistently elicited antennal responses from male PAM antennae (Figure 2). By comparing mass spectra and retention indices (Van den Dool and Kratz, 1963) of PAM-produced compounds with those of our reference library of moth pheromones (RG & GG, unpublished), and by comparative GC, GC-MS, and GC-EAD analyses of PAM-produced components and authentic standards on three GC columns (DB-5, DB-23, DB-210), we determined

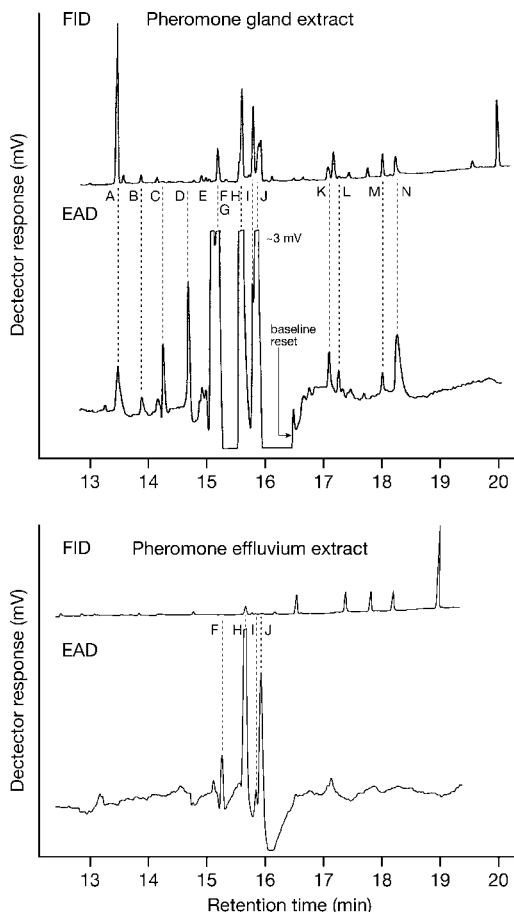


FIG. 2. Flame ionization detector (FID) and electroantennographic detector (EAD: male *Teia anartoides* antenna) responses to 0.1 female equivalent (FE) of pheromone gland extract of female *T. anartoides* (top), or to 30 female-hour equivalents (FHE = all volatiles released by 30 females during 1 hr of aeration) of Porapak Q extract (bottom). Chromatography: DB-5 column; splitless injection; temperature program: 100°C/1 min, 10°C per min to 280°C. **A** = (Z,Z)-6,9-heneicosadiene (Z6Z9-21Hy); **B** = heneicosatriene (with unknown double bond positions); **C** = (Z6)-*cis*-9,10-epoxy-eicosene (Z6-9,10-epo-20Hy); **D** = (Z,Z)-6,9-eicosadiene-11-one (Z6Z9-11-one-20Hy); **E** = (Z,Z)-6,9-heneicosadien-11-ol (Z6Z9-11-ol-21Hy); **F** = (Z6)-*cis*-9,10-epoxy-heneicosene (Z6-9,10-epo-21Hy); **G** = (Z)-6-heneicosen-11-one (Z6-11-one-21Hy); **H** = (Z,E)-6,8-heneicosadien-11-one (Z6E8-11-one-21Hy); **I** = (Z,E)-6,9-heneicosadien-11-one (Z6E9-11-one-21Hy); **J** = (Z,Z)-6,9-heneicosadien-11-one (Z6Z6-11-one-21Hy); **K** and **L** = unknown rearrangement products of Z6Z9-11-one-21Hy; **M** = (E,E)-7,9-heneicosadien-6,11-dione (E7E9-6,11-dione-21Hy); **N** = 6-hydroxy-(E,E)-7,9-heneicosadien-11-one (E7E9-6-ol-11-one-21Hy).

that components **A-J** were Z6Z9-21Hy (**A**), a heneicosatriene with undetermined double bonds (**B**), Z6-9,10-epo-20Hy (**C**), Z6Z9-11-one-20Hy (**D**), Z6Z9-11-ol-21Hy (**E**), Z6-9,10-epo-21Hy (**F**), Z6-11-one-21Hy (**G**), Z6E8-11-one-21Hy (**H**), Z6E9-11-one-21Hy (**I**), and Z6Z9-11-one-21Hy (**J**). Although labile Z6Z9-11-one-21Hy rearranges to Z6E8-11-one-21Hy during GC analyses (Gries et al., 1997; Wimalaratne, 1998), trace amounts of Z6Z9-11-one-21Hy remained detectable by PAM antennae (Figure 2), suggesting that pheromone extracts may contain larger quantities of this labile ketodiene than are evident from Figure 2.

To determine unequivocally the presence and ratio of the three ketodienes (Z6Z9-11-one-21Hy, Z6E8-11-one-21Hy, Z6E9-11-one-21Hy) in pheromone gland extracts, extracts were analyzed by HPLC-MS, a procedure that minimized isomerization of labile ketodienes. Comparative HPLC-MS analyses of synthetic Z6Z9-11-one-21Hy and of PAM pheromone extract revealed that the most abundant (~57 ng per FE) candidate pheromone component in PAM pheromone extracts eluted at the same time as synthetic Z6Z9-11-one-21Hy (Figure 3). Identical HPLC retention times (Figure 3), mass spectra (Figure 3), and UV spectra (Figure 4) of this component and of synthetic Z6Z9-11-one-21Hy confirmed that Z6Z9-11-one-21Hy was present in relatively large quantities in PAM pheromone extracts. Z6E8-11-one-21Hy and Z6E9-11-one-21Hy were also present but at lower quantities (Figure 3).

The absolute configurations of candidate pheromone components Z6Z9-11-ol-21Hy (**E** in Figure 2) and Z6-9,10-epo-21Hy (**F** in Figure 2) were determined by HPLC fractionation of PAM extract (20 FE), followed by GC analyses of concentrated (2 μ l) HPLC fractions and authentic standards on a chiral stationary phase GC column. These analyses revealed that PAM females produce both enantiomers (1:1) of Z6Z9-11-ol-21Hy (data not shown), and the (9*R*,10*S*)-enantiomer of Z6-9,10-epo-21Hy (Figure 5).

Mass spectra and molecular weights of late-eluting components **M** and **N** (Figure 6) suggested that they were heneicosadienes with two oxygens. Considering that PAM females produce epoxides and ketones (Figure 2), we hypothesized that compound **M** (MW = 320) was an epoxy-ketone or diketone, and compound **N** (MW = 322) an epoxy- or keto-alcohol. Chromatographic "tailing" of **N** (Figure 2) suggested the presence of a hydroxy-group in **N**. Hydrogenation of the **M**- and **N**-containing HPLC fraction, and GC-MS analyses of the hydrogenation products, produced a new mass spectrum with fragmentation ion m/z 169 indicative of a carbonyl group on C11. To test the hypothesis that **M** and **N** were biosynthetically related to Z6Z9-11-one-21Hy or Z6E8-11-one-21Hy, and to confirm the presence of a second oxygen in **M** and **N**, we epoxidized and hydrogenated synthetic Z6E8-11-one-21Hy. One of the resulting compounds had the same retention time and mass spectral characteristics as the **M** hydrogenation product. Bearing in mind that epoxidation and hydrogenation of Z6E8-11-one-21Hy could afford both epoxy-ketones and diketone by-products (Malinovskii, 1965), we further hypothesized

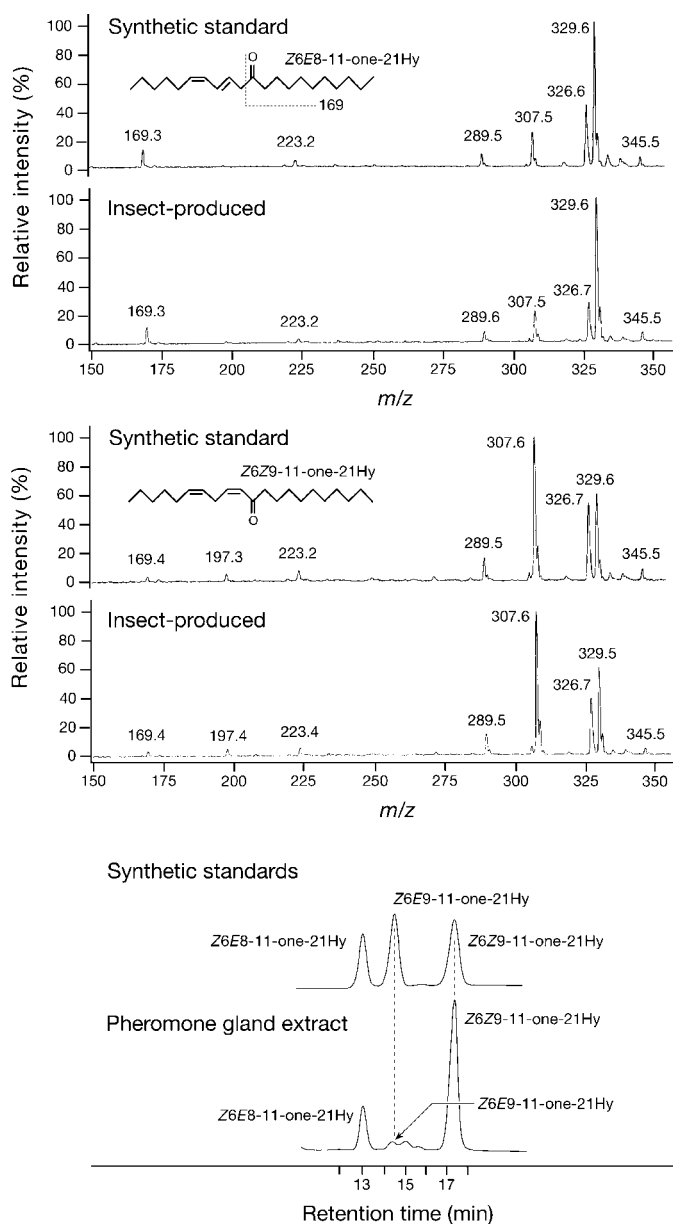


FIG. 3. (Top). High-performance liquid chromatography atmospheric pressure electrospray (APES) mass spectra (200 volts) of synthetic and insect-produced (Z,E)-6,8-heneicosadien-11-one (Z6E8-11-one-21Hy; ~ 10 ng/ μ l), and of (Z,Z)-6,9-heneicosadien-11-one (Z6Z9-11-one-21Hy; ~ 10 ng/ μ l); (Bottom) HPLC chromatograms of synthetic standards and pheromone gland extract of female *T. anartoides*.

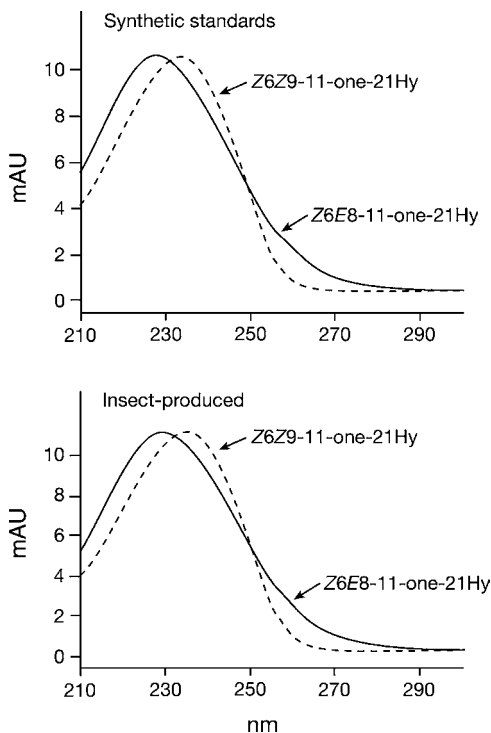


FIG. 4. UV/Visible spectra of synthetic and insect-produced (*Z,E*)-6,8-heneicosadien-11-one (Z6E8-11-one-21Hy) and (*Z,Z*)-6,9-heneicosadien-11-one (Z6Z9-11-one-21Hy). Injection volume on HPLC: 0.5 μ l with \sim 5 ng per compound on column. Note contrasting absorption characteristics and peak maxima (234 nm and 228 nm) for Z6E8-11-one-21Hy and Z6Z9-11-one-21Hy.

that the second oxygenated group in **M** was either a *cis*-6,7- or *trans*-8,9-epoxide, or, a ketone at C6, C7, C8, or C9. Heneicosan-6,11-dione as our first synthetic candidate had the same mass spectrum and retention times on three columns as the **M** hydrogenation product. In assigning the double bond positions to the target heneicosadien-6,11-dione (**M** in Figure 2), we considered (a) known double bond positions (*Z*6, *E*8, *Z*9, *E*9) in ketodienes from *Orgyia* spp. (Gries et al., 1997; Lui, 1999; Grant et al., 2003), and (b) the large retention indices of **M** (DB-5: 2586, DB-210: 3275) that were indicative of extensive conjugation. (*E,E*)-7,9-Heneicosadien-6,11-dione was synthesized as the thermodynamically most stable target and was shown to have the same mass spectral (Figure 6) and retention characteristics as **M** in Figure 2. Moreover, the corresponding 6-hydroxy-(*E,E*)-7,9-heneicosadien-11-one met all identification criteria of component **N** in Figure 2.

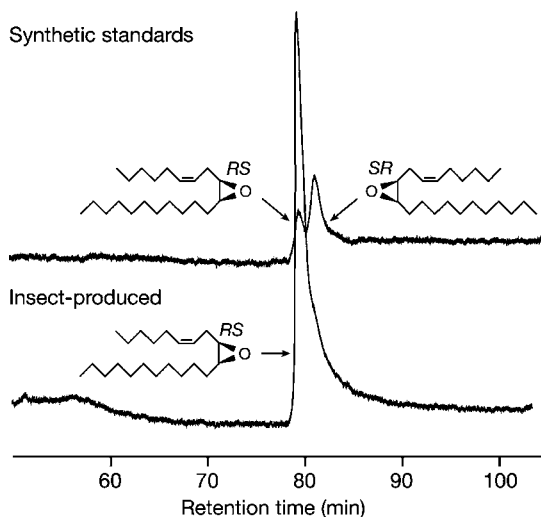


FIG. 5. Chromatogram of a mixture of synthetic enantiomers of (Z)-6-*cis*-9,10-epoxy-heneicosene (*top*) and of insect-produced (Z6,9*R*,10*S*)-*cis*-9,10-epoxy-heneicosene (50 female equivalents) (*bottom*) on a chiral stationary phase GC column.

Its mass spectrum is shown in Figure 6. Identifications of **K** and **L** were not attempted because both compounds were rearrangement products of Z6Z9-11-one-21Hy, suggesting that they are not likely part of the PAM pheromone.

GC-EAD analyses of effluvia of calling PAM females (Figure 2) revealed significant antennal responses to Z6-9,10-epo-21Hy, Z6Z9-11-one-21Hy, and Z6E8-11-one-21Hy (which forms from Z6Z9-11-one-21Hy during GC analyses), suggesting that the actual pheromone blend might be much simpler than suggested by the pheromone gland extracts.

Behavioral Bioassays. In preliminary wind tunnel bioassay experiments, attraction of male moths to Z6Z9-11-one-21Hy (the major candidate pheromone component) appeared enhanced by specific HPLC fractions of pheromone gland extracts, or corresponding synthetic candidate pheromone components. Besides Z6Z9-11-one-21Hy, three components (Z6-9*R*10*S*-epo-21Hy, E7E9-6,11-dione-21Hy, and E7E9-6-ol-11-one-21Hy) seemed to affect the males' responses and were bioassayed as blends in experiments 1 and 2 (Table 1). When all three of these components were added to Z6Z9-11-one-21Hy, more PAM males appeared to orient towards the lure, flew midway through the wind tunnel, approached, and contacted the lure (Table 1).

In field trapping experiment 1 in Australia, Z6Z9-11-one-21Hy as a single component attracted PAM males. Moreover, experiment 1 suggested that the

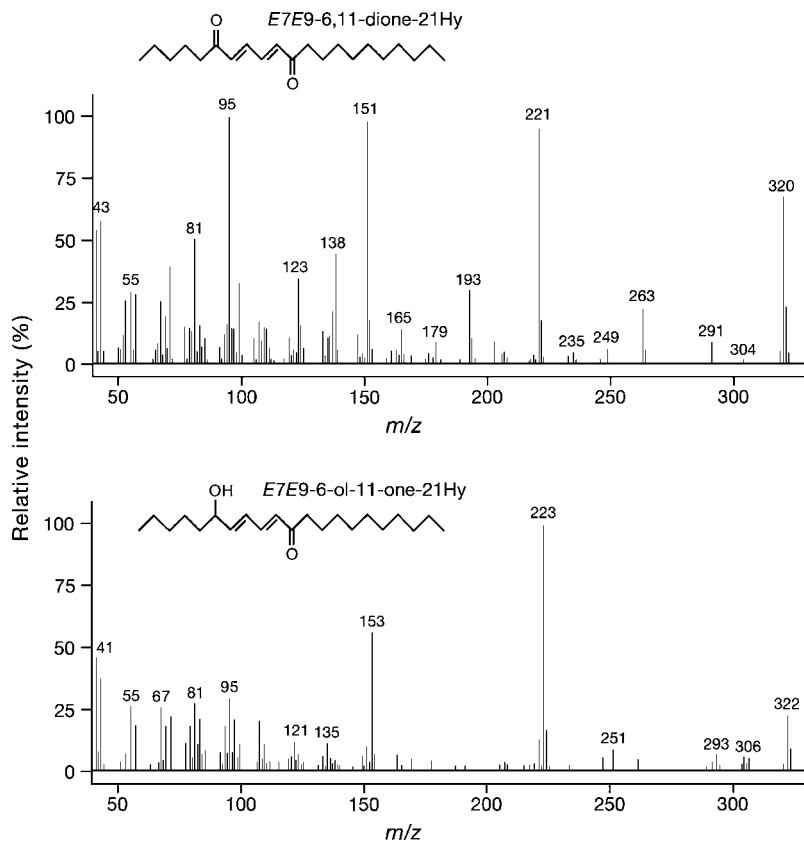


FIG. 6. Ion trap mass spectra of (*E,E*)-7,9-heneicadien-6,11-dione (*E7E9-6,11-dione-21Hy*) and 6-hydroxy-(*E,E*)-7,9-heneicosen-11-one (*E7E9-6-ol-11-one-21Hy*) identified in pheromone gland extracts of female *Teia anartoides*. Synthetic standards gave the same spectra.

4-component blend of Z6Z9-11-one-21Hy, Z6-9R10S-epo-21Hy, *E7E9-6,11-dione-21Hy*, and *E7E9-6-ol-11-one-21Hy* at a 5:0.5:0.5:0.5 ratio might attract more PAM males than Z6Z9-11-one-21Hy alone. The 4-component blend at a 5:5:5:5 ratio was not attractive (Figure 7). However, experiments 2–4 failed to demonstrate conclusively whether any of the minor components, singly or in combinations, increased the attractiveness of blends relative to Z6,Z9-11-one-21Hy alone (Figures 7 and 8). In experiment 5, traps baited with Z6Z9-11-one-21Hy at 5 μ g were more attractive than unbaited control traps, whereas Z6 E8-11-one-21Hy and Z6-9R10S-epo-21Hy (5 μ g each) were not different from controls (data not shown).

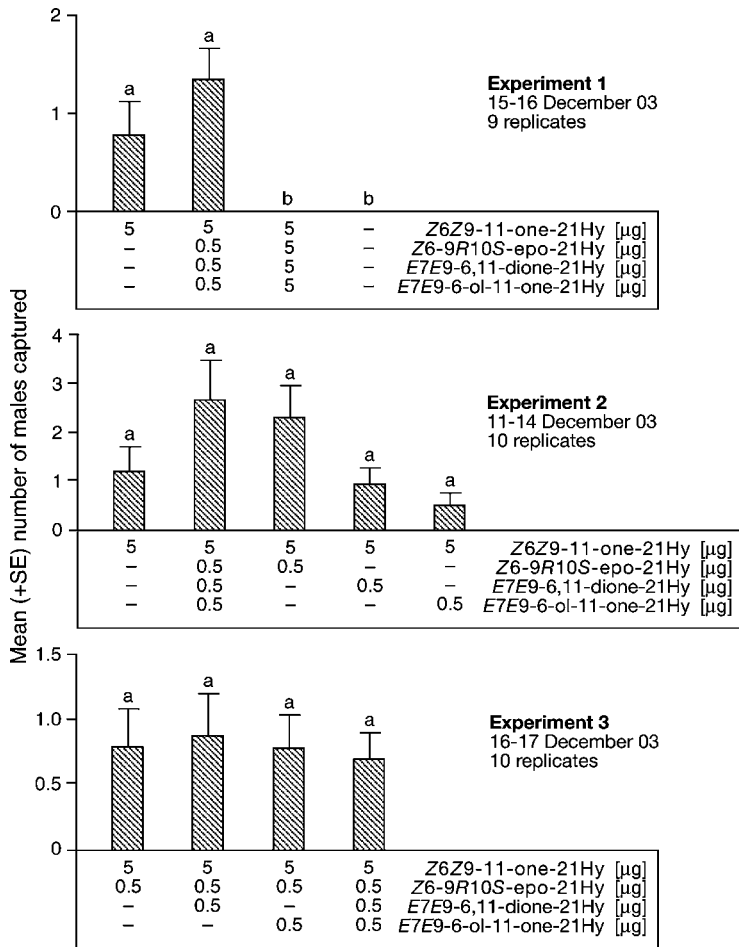


FIG. 7. Captures of male *Teia anartoides* in field experiments 1–3 in sticky traps baited with candidate pheromone components singly and in combinations; all experiments near Campbelltown, N.S.W., Australia. In each experiment, bars with the same letter superscript are not significantly different; $\alpha = 0.05$. Compound abbreviations as in Figure 2.

DISCUSSION

Z6Z9-11-one-21Hy appears to be the major sex pheromone component of PAM, on the basis of (1) analyses of pheromone gland extracts of PAM females by GC-EAD, GC-MS, HPLC-MS, and HPLC-UV/Visible spectroscopy; (2) GC-EAD analyses of effluvia of PAM females; and (3) wind tunnel and field experiments

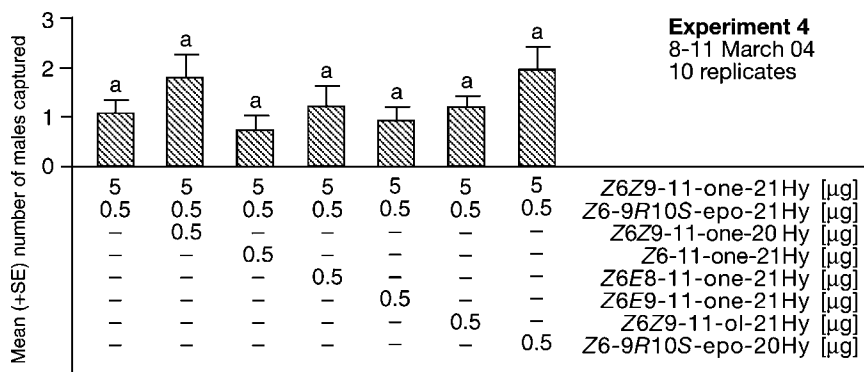


FIG. 8. Captures of male *Teia anartoides* in field experiments 4 in sticky traps baited with candidate pheromone components in various combinations; all experiments near Campbelltown, N.S.W., Australia. Bars with the same letter superscript are significantly different; $\alpha = 0.05$. Compound abbreviations as in Figure 2.

with a synthetic standard. The same compound has been reported as the major sex pheromone component of whitemarked tussock moth, *Orgyia leucostigma* (Liu, 1999; Grant et al., 2003). Whether Z6E8-11-one-21Hy is a (secondary) pheromone component of PAM females (Jury et al., 2003) is not easily determined because lures loaded with Z6Z9-11-one-21Hy as a single component will, almost immediately, also release the rearrangement product Z6E8-11-one-21Hy, thus complicating comparison of such lures with those baited from the beginning with both Z6Z9-11-one-21Hy and Z6E8-11-one-21Hy.

HPLC-MS was crucial in confirming the presence and ratio of Z6Z9-11-one-21Hy, Z6E8-11-one-21Hy, and Z6E9-11-one-21Hy in pheromone gland extracts. Aqueous methanol (90% MeOH) provided optimal reverse phase chromatographic separation between Z6E8-11-one-21Hy and Z6Z9-11-one-21Hy, with retention times reproducible within 0.1 min between runs. Among atmospheric pressure electrospray (APES), atmospheric pressure chemical ionization (APCI), and atmospheric pressure photo ionization (APPI), APES provided the greatest sensitivity and most readily interpretable mass spectra. No APES mass spectra were obtained with acetonitrile as the solvent system. Changes in nebulizer gas temperature from 200°C to 350°C reduced sensitivity but did not result in changes to the mass spectra. A 150-volt fragmentation voltage revealed the major ions 307.5 amu (MH^+) and 329.5 amu (MNa^+) for both compounds, but at 200 volts (Figure 3) the fragmentation ion 169.3 amu and adduct ion 326.5 amu were particularly visible. The fragment ion m/z 169.3 is also observed in corresponding GC-MS analyses (data not shown).

In wind tunnel experiments (Table 1), the 4-component blend of Z6Z9-11-one-21Hy, Z6-9R10S-epo-21Hy, E7E9-6,11-dione-21Hy, and E7E9-6-ol-11-one-21Hy appeared more attractive to males than Z6Z9-11-one-21Hy as a single component. However, the biological activity of the former three components was not confirmed in field experiments, possibly as a result of inappropriate blend ratios, release rates, or trap designs. It remains to be determined whether or not Z6-9R10S-epo-21Hy, E7E9-6,11-dione-21Hy, and E7E9-6-ol-11-one-21Hy are indeed PAM sex pheromone components. The epoxide Z6-9R10S-epo-21Hy is the most likely candidate for a secondary pheromone component because it was present in the effluvia of calling females (Figure 2), and appeared to enhance attractiveness of Z6Z9-11-one-21Hy in field experiment 2 (Figure 7).

Attractiveness of synthetic pheromone lures and of virgin PAM females also will have to be compared in field trials to determine whether synthetic lures could replace PAM females as a trap bait in the program to monitor eradication of PAM in New Zealand.

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